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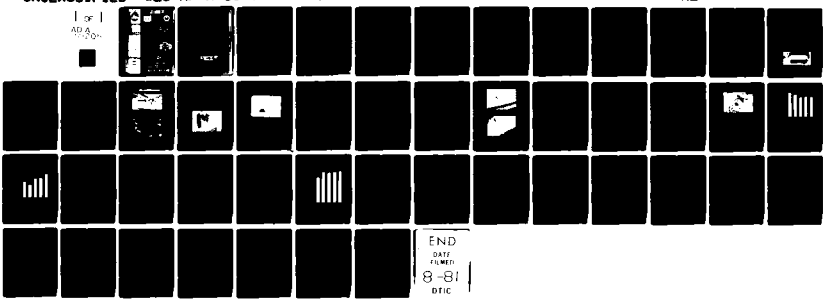

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EVALUATION OF A FORMULATION OF CERCOSPORA RODMANII FOR INFECTIVITY AND PATHOGENICITY OF WATERHYACINTH

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As a part of a Large-Scale Operations Management Test (LSOMT) of insects and pathogens for the control of waterhyacinth in Louisiana, a formulation of the fungal pathogen Cercospora rodmanii was preliminarily evaluated to determine application rates required to achieve adequate infection of waterhyacinth under environmental conditions normally found in Louisiana. In a spring study, a C. rodmanii formulation containing 1×10^6 viable propagules per gram was applied to test tanks at rates of 0, 5, 10, and 20 g/m^2. After (Continued)

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6 weeks, it was concluded that a treatment rate of 5×10^6 viable propagules per square metre provides adequate infection of waterhyacinth in a spring application. In a fall study, treatment rates of 0, 1, 2.5, and 10 g/m^2 of the C. rodmanii formulation containing 4×10^6 viable propagules per gram were applied to replicated test tanks. After 6 weeks, all tanks that received C. rodmanii were infected by the fungus, but there were no significant differences in the level of infection among C. rodmanii treatments.

Therefore, a rate of 4×10^6 viable propagules per square metre was deemed sufficient for a fall application. In both studies, significant secondary infection of waterhyacinth tissues was observed on new leaves of original plants and on daughter plants. Thus, progressive pathogenesis can be expected to occur when field populations of waterhyacinth are infected by C. rodmanii. Based on the results of these studies, rates of 5×10^6 and 4×10^6 viable propagules per square metre will be used for large-scale applications of C. rodmanii in spring and fall tests, respectively.

PREFACE

Funds for the studies described herein were provided to the U. S. Army Engineer Waterways Experiment Station (WES) Aquatic Plant Control Research Program (APCRP) through the Department of the Army Appropriation No. 96X3123, "Operations and Maintenance General," by the U. S. Army Engineer District, New Orleans.

This report describes a study to determine treatment rates to be used in large-scale evaluations of the fungal pathogen _Cercospora rodmanii_ as part of the Large-Scale Operations Management Test (LSOMT) of insects and pathogens for the control of waterhyacinth in Louisiana. Dr. D. R. Sanders, Sr., was the team leader for the LSOMT. Mr. R. F. Theriot was responsible for field application tests, and Mr. E. A. Theriot was responsible for the _Cercospora_ efficacy tests.

This report was prepared by Messrs. E. A. Theriot and R. F. Theriot and Dr. Sanders of the Wetland and Terrestrial Habitat Group (WTHG), Environmental Resources Division (ERD), Environmental Laboratory (EL), WES. Mr. Samuel O. Shirley (WTHG) established the test tanks and assisted in the collection of data. Mr. David L. Leese, Instrumentation Services Division, WES, established and maintained the weather station used in the study. Abbott Laboratories, Inc. (AL), provided the _Cercospora_ formulation evaluated in the study and Dr. Donald S. Kenney of AL offered valuable technical assistance.

All phases of this study were conducted under the direct supervision of Dr. H. K. Smith, Acting Chief, WTHG, and under the general supervision of Dr. C. J. Kirby, Jr., Chief, ERD, and Dr. John Harrison, Chief, EL. Manager of the APCRP at the WES was Mr. J. L. Decell.

Commanders and Directors of the WES during the performance of the research and preparation of the report were COL John L. Cannon, CE, and COL Nelson P. Conover, CE. Technical Director was Mr. F. R. Brown.

This report should be cited as follows:

> Theriot, E. A., Theriot, R. F., and Sanders, D. R., Sr. 1981. "Evaluation of a Formulation of _Cercospora rodmanii_ for Infectivity and Pathogenicity of Waterhyacinth," Technical Report A-81-5, U. S. Army Engineer Waterways Experiment Station, CE, Vicksburg, Miss.

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EVALUATION OF A FORMULATION OF *CERCOSPORA RODMANII* FOR INFECTIVITY AND PATHOGENICITY OF WATERHYACINTH

PART I: INTRODUCTION

Background

1. In December 1973, Dr. K. E. Conway of the University of Florida, while performing research for the Corps' Aquatic Plant Control Research Program (APCRP), isolated a plant pathogen associated with declining waterhyacinth (*Eichhornia crassipes* (Mart.) Solms.) in Rodman Reservoir, Fla. (Conway, Freeman, and Charudattan 1974). Taxonomic studies revealed this fungal organism to be a new species of *Cercospora* that Conway (1976a) named *Cercospora rodmanii* Conway (Form Class: Fungi Imperfecti).

2. In subsequent studies, *C. rodmanii* was found to be suitably host specific for use as a biocontrol agent of waterhyacinth (Conway and Freeman 1977). Test results were so encouraging that the University of Florida negotiated with Abbott Laboratories, Chicago, Ill., for the experimental mass production of *C. rodmanii* inoculum for large-scale field trials. Subsequently, the University of Florida was granted a patent for the use of *C. rodmanii* as a waterhyacinth control agent. The university then granted Abbott Laboratories the right to develop a product form for sale and distribution.

3. The U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss., is presently conducting a Large-Scale Operations Management Test in Louisiana for the control of waterhyacinth using insects and pathogens (LSOMT-IP) (Sanders et al. 1979). Abbott Laboratories' formulation of *C. rodmanii* will be tested alone and in combination with insects to control waterhyacinth. The project is being funded by the U. S. Army Engineer District, New Orleans. The New Orleans District has had a continuing problem with waterhyacinth since shortly after its introduction into this country in 1884 (Klorer 1909).

Rationale

4. Abbott Laboratories has developed a wettable powder formulation of *C. rodmanii*. However, the treatment rate and optimal time of year for application required to achieve adequate infection, pathogenicity, and control of waterhyacinth are not known (Theriot, Sanders, and Theriot 1981). It was determined that such data gaps must be filled to successfully conduct the LSOMT-IP.

5. The optimal temperature range for the growth of *C. rodmanii* is 20°-30° (Conway and Freeman 1976; Freeman, Charudattan, and Conway 1981). To ensure the best possible conditions for disease development, the formulation should be applied at the time of year when the greatest portion of a 24-hr day is within that temperature range. These temperature requirements are best satisfied in the early spring and late fall in Louisiana. Subsequently, studies were initiated at the WES to evaluate the infectivity and pathogenicity of the *C. rodmanii* formulation under environmental conditions that occur in the field during early spring and late fall.

Purpose and Objectives

6. The purpose of the preliminary, small-scale outdoor tests was to evaluate the viability, infectivity, and pathogenicity of the *C. rodmanii* formulation.

7. The objectives of the tests were:

a. To evaluate the infectivity and pathogenicity of the *C. rodmanii* formulation on waterhyacinth.

b. To determine effects of *C. rodmanii* on the vegetative reproduction of waterhyacinth.

c. To determine the optimum season of the year and environmental conditions for infection of *C. rodmanii* and disease development on waterhyacinth.

d. To establish treatment rates to be used in applications scheduled for the LSOMT-IP in Louisiana.

e. To obtain information for inclusion in a manual for the operational use of this *C. rodmanii* formulation as a biocontrol agent of waterhyacinth.

PART II: SPRING STUDY

Procedure

Experimental units

8. Fifteen tanks (0.6 by 1.8 by 0.8 m) (Figure 1) were established on the WES reservation on 15 March 1979 (Table 1). Five tanks were placed in each of three areas, and individual tanks were separated by a minimum of 100 m to minimize cross-contamination (Figure 2). To prevent a toxic reaction by the waterhyacinths to the zinc lining in the walls of the galvanized tanks, each tank was lined with a double layer of polyethylene. The tanks were filled with tapwater to within 3 cm of the top and maintained at that level throughout the test period. The pH was adjusted to 6.5 in each tank prior to application of C. rodmanii and was maintained within the range of 6.0 to 7.0 throughout the study by adding the required amount of sodium bicarbonate and phosphoric acid.

9. Nutrient solution was added to each tank (Table 1) to ensure that the growth of waterhyacinth was not limited by nutrient deficiencies. Two hundred fifty millilitres (312 mg/ℓ) of liquid nutrients (12-6-6) was added to each tank before application and an additional 100 ml (125 mg/ℓ) was added to the tanks 14 days posttreatment (Table 1).

10. Twelve waterhyacinth plants were placed in each tank on 19 March 1979 (Table 1). These plants were propagated in the greenhouse,

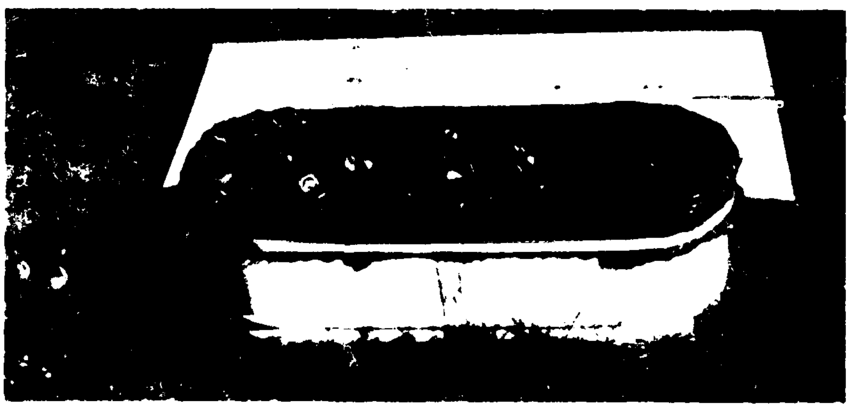

Figure 1. Experimental test tank used in the study

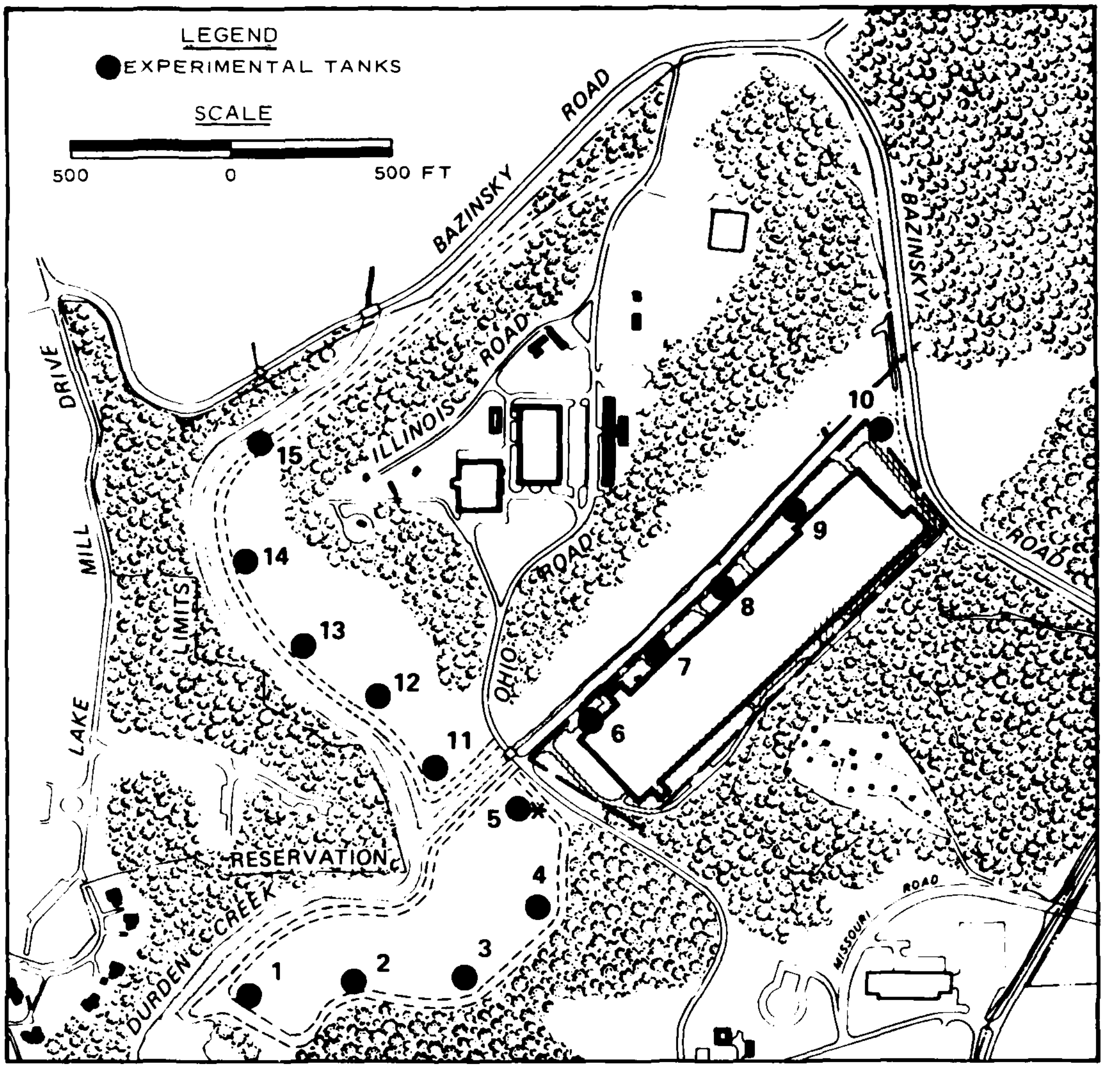

Figure 2. Map of the northern portion of the WES reservation showing the locations of experimental tanks. (Weather station located at tank 5)

and were vigorous and disease-free when placed in the tanks. Plants were selected to simulate the small, bulbous-petioled plants likely to be encountered in the field during a spring application. All senescent leaves and all daughter plants were removed before the plants were added to the tanks.

Pretreatment data collection

11. On the day prior to treatment application (Table 1), six plants were randomly selected from each tank, the root lengths and biomass (wet weight) were measured to estimate these parameters for the remaining plants, and the plants were discarded. The six plants remaining in each tank constituted the test population. The small number of waterhyacinth plants precluded crowding and allowed space for the production of daughter plants from stolons. In this manner, it was possible to evaluate the ability of C. rodmanii to limit vegetative reproduction of waterhyacinth.

12. The newest emergent leaf of each plant was tagged on the day of treatment application (Figure 3). This enabled discrimination between the original plant tissue that received direct application of the pathogen and new, untreated tissue. The original tissue of each plant consisted of the tagged leaf and all leaves distal to it, while the new plant growth consisted of all plant tissues proximal to the tagged leaf, and all daughter plants.

Formulation and application

13. Abbott Laboratories developed a formulation of C. rodmanii for use in large-scale applications. The organism was incorporated in a wettable powder matrix for application by conventional herbicide application systems (Theriot, Sanders, and Theriot 1981). The process used to produce the formulation was known only to staff members of Abbott Laboratories and was of a proprietary nature.

14. The viable propagules of C. rodmanii in the formulation were characterized as thick-walled vegetative cells (Figure 4). To facilitate application, the dried inoculum was milled sufficiently to pass through a No. 24 mesh screen. The formulation had a shelf life of approximately 6 months.*

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* Personal Communication, Oct 1979, Dr. Donald Kenney, Abbott Laboratories, Chicago, Ill.

Figure 3. Procedure for tagging the newest leaf on the original plants used in the study

Figure 4. Microscopic view of *C. rodmanii* propagules in Abbott Laboratories' formulation (×200)

15. The formulation of C. rodmanii, containing 1×10^6 CFU* per gram, was applied at rates of 5, 10, and 20 g/m^2 on 13 April 1979 (Table 1). In addition, there were two sets of controls: one sprayed with the carrier substrate contained in the formulation, and an untreated control. Each treatment rate and control was replicated three times.

16. Treatments were randomly allocated to test tanks within each test area, and a hand-held sprayer (Figure 5) was used to apply the formulation of C. rodmanii at the rates specified in paragraph 15. Because there was a threat of rain, immediately following application each tank was covered with a sheet of plywood for a 24-hr period to ensure that the inoculum would remain on the leaf surface for sufficient time to initiate infection.

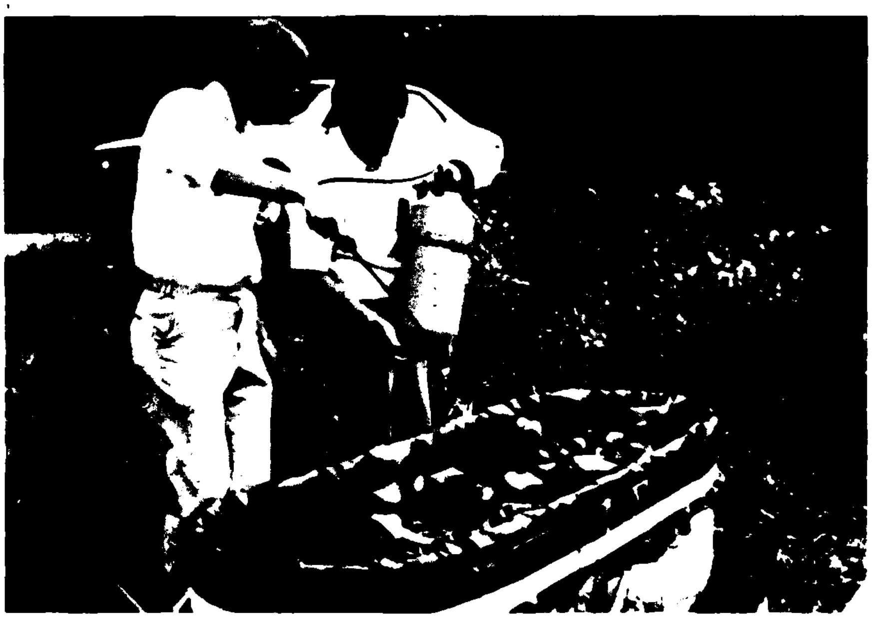

Figure 5. Application of C. rodmanii formulation to the test plants

Posttreatment data collection

17. Physical parameters. To monitor environmental conditions

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* CFU = Colony Forming Unit.

that could affect the results of the study, an automated weather station was established at tank 5 (Figure 6). Ambient air temperature at 60 cm above the canopy, and air temperature and relative humidity in the waterhyacinth canopy, were monitored on an hourly basis throughout the test period.

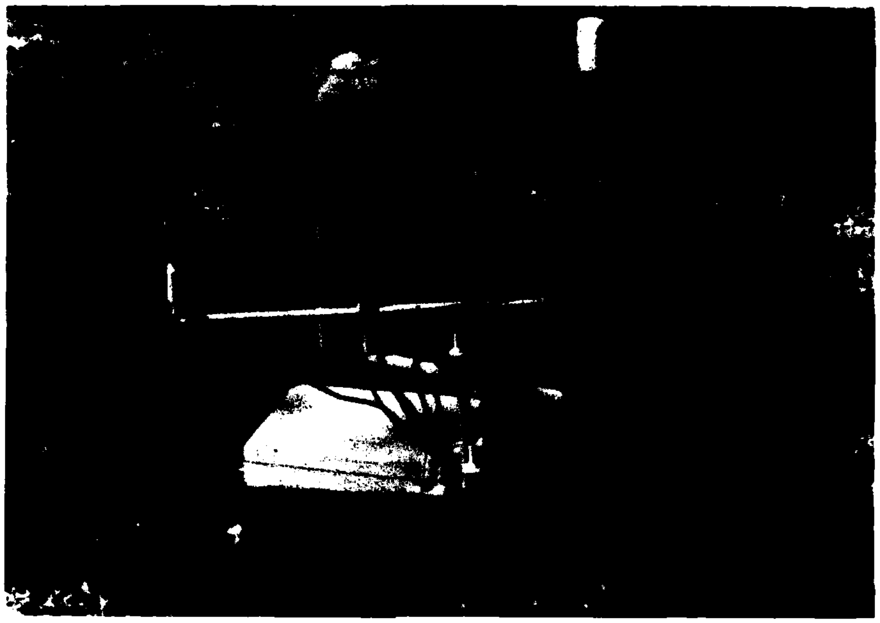

Figure 6. Weather station used to record air temperature and relative humidity during the spring study

18. Biological parameters. All plants in each tank were examined on each sampling date (Table 1). Data collected from each tank included disease damage per leaf for both original and new plant tissues, number of new leaves per plant, number of dead leaves per plant, height of the original plant, and total number of daughter plants per tank. Root lengths and biomass (wet weight) were recorded on the last sampling date. Both color and infrared photographs were taken of each tank on each sampling date.

19. Damage per leaf was recorded using a modification of the rating scale developed by Dr. Conway and associates at the University of Florida (Table 2) (Conway and Freeman 1976). Damage was rated on a

scale of 1 to 10 where 1 represented no apparent infection of the leaf and 10 indicated a dead submerged leaf blade and petiole. Intermediate values corresponded to increasing coverage of the leaf blade by the disease symptoms. However, the values for disease damage per leaf reflected not only symptoms produced by *C. rodmanii*, but also disease symptoms produced by facultative pathogens.

Data analysis

20. The statistical model used to analyze the data was a one-way analysis of variance. The Statistical Analysis System (SAS), version 76.6D, was used to perform the statistical analyses. Duncan's Multiple Range Test was used to test for significant differences between treatment means.

Results

Physical parameters

21. Air temperatures. Air temperatures in the waterhyacinth canopy averaged 2.5°C higher than the ambient air temperatures during the first 24 hr after treatment (Figure 7). The average ambient air temperature for that period was 16.6°C with a range from 7.4° to 27.9°C, while the air temperature within the waterhyacinth canopy averaged 19.1°C with a range from 12.3° to 27.6°C. Therefore, use of the covers resulted in significantly higher, more favorable temperatures for infection and growth of *C. rodmanii* during the late evening and early morning hours. After the covers were removed, no significant difference occurred between the ambient air temperatures and the air temperatures in the plant canopy for the duration of the test.

22. Air temperatures in the waterhyacinth canopy were within the temperature range favorable to growth of *C. rodmanii* approximately 41 percent of the initial 3-day infection period (Figure 8), 42 percent of the first 7 days, 44 percent of the period from day 7 to day 14, 34 percent of the period from day 14 to day 28, and 52 percent of the final 10 days of the test. For the study period, ambient temperatures in the waterhyacinth canopy were within the favorable temperature range

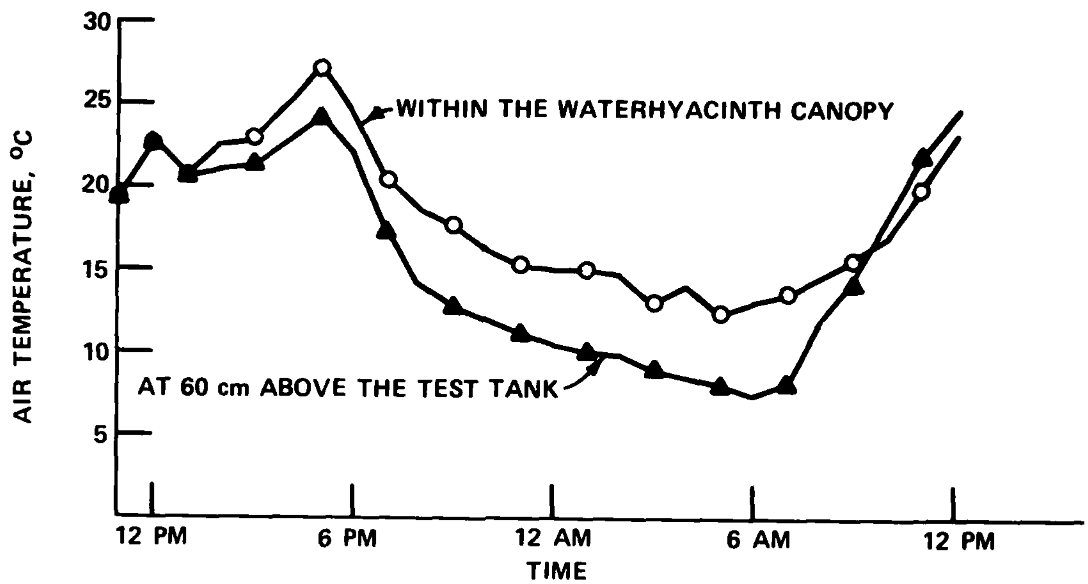

Figure 7. Comparison of ambient temperatures in, and at 60 cm above, the waterhyacinth canopy during the initial 24-hr period following application

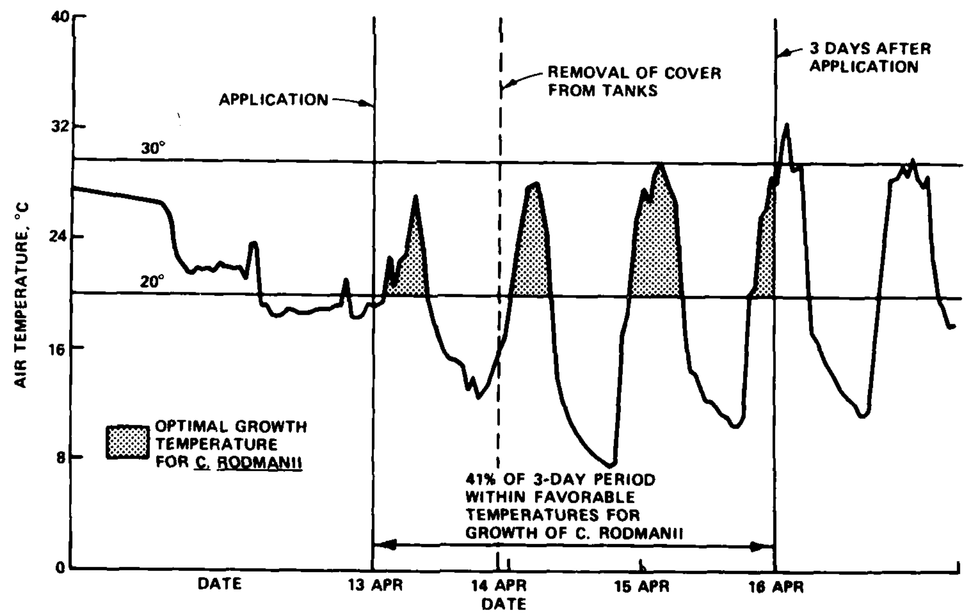

Figure 8. Air temperatures within the waterhyacinth canopy during the first 3 days after application

for the growth of C. rodmanii 41 percent of the time.

23. Relative humidity. Relative humidity in the waterhyacinth canopy averaged 95 percent for the initial 24-hr period. These relative humidity values were optimal for germination of the propagules and infection and were never below 70 percent during the entire study (Figure 9).

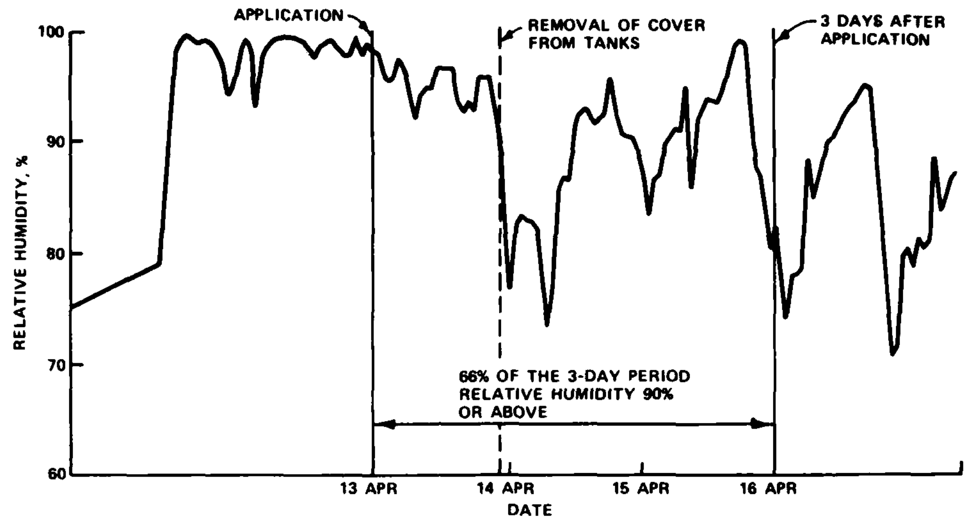

Figure 9. Relative humidity within the waterhyacinth canopy during the first 3 days after application

Biological parameters

24. Growth of control plants. An average of nine new leaves was produced on each of the original plants in the control tanks during the 6-week test period. The originally treated leaves died and were completely replaced after 6 weeks. The average number of daughter plants per tank was 174, or approximately 29 per original plant. The average biomass (wet weight) increased from 0.97 to 14.15 kg per tank and the root lengths from 17.2 to 18.3 cm per plant. The average height per plant increased from 13.5 to 30.3 cm (125 percent) during the study. These data demonstrated the rapid reproductive and growth potential of waterhyacinth (Table 3).

25. Infectivity. Three days after treatment, reddish-brown blemishes averaging 3 mm in diameter occurred on the leaf surface around the particles of formulation on plants in treated tanks (Figures 10 and 11).

Figure 10. Surface of treated waterhyacinth leaf 3 days after application. Reddish-brown areas represent infection loci

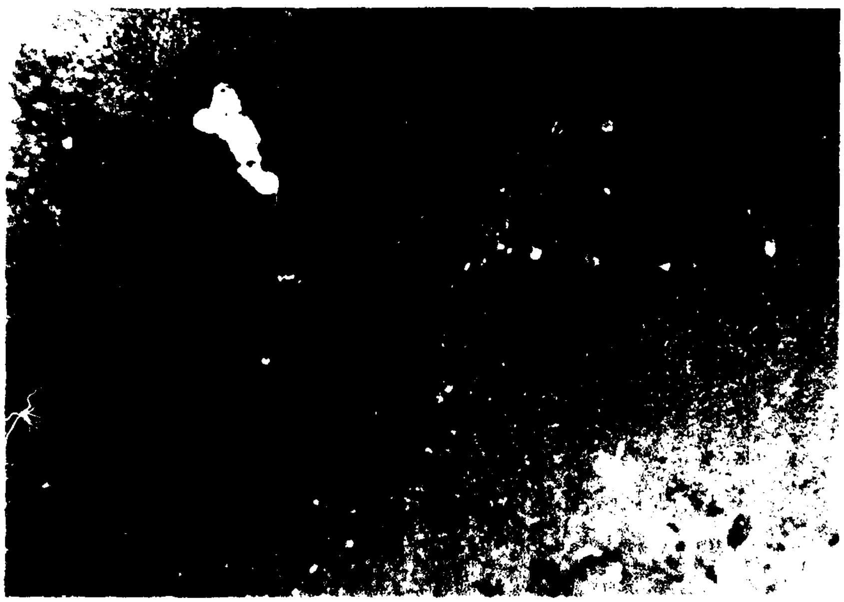

Figure 11. Closeup of treated waterhyacinth leaf 3 days after application. Dark areas represent infection loci; formulation particles are visible on the surface

At that time, ADI* values were statistically significantly higher in treated tanks than in either set of controls (Table 4). The higher ADI values were a direct result of the blemishes on the leaf surfaces in the treated tanks. No daughter plants or dead leaves were recorded at 3 days posttreatment, and there were no significant differences in numbers or ADI values of new leaves. Slightly higher ADI values occurred in tanks treated with the carrier substrate than in the untreated control tanks. The carrier substrate apparently served as a supplemental nutrient source for ubiquitous saprophytes and facultative pathogens on the leaf surfaces.

26. Pathogenicity. Typical *C. rodmanii* disease symptoms (punctate leaf spots) became evident 2 weeks after application and continued to increase in severity in the treated tanks throughout the 6 weeks of the study. The ADI values were as follows:

a. Original tissue. Higher ADI values were recorded for original tissues in the treated tanks than for the control tanks during the study period (Figure 12). However, the ADI values for treated plants were statisically significantly higher than for controls only on the 3-, 7-, and 14-day sampling dates (Table 4). The ADI values were not significantly different for the last two sampling dates because nearly all originally treated leaves had been replaced on all plants by the 28-day sampling date. Although the ADI values for plants in tanks treated with the higher rates of the formulation were consistently higher than ADI values for plants in tanks treated with the lower rates, there were no significant differences between treatment rates. Therefore, a treatment rate of 5 g/m^2 (5×10^6 CFU/m^2) was considered to be sufficient for achieving infectivity in a spring application.

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* ADI = Average Disease Index per leaf. These values were obtained by dividing total disease ratings per plant by the number of leaves per plant.

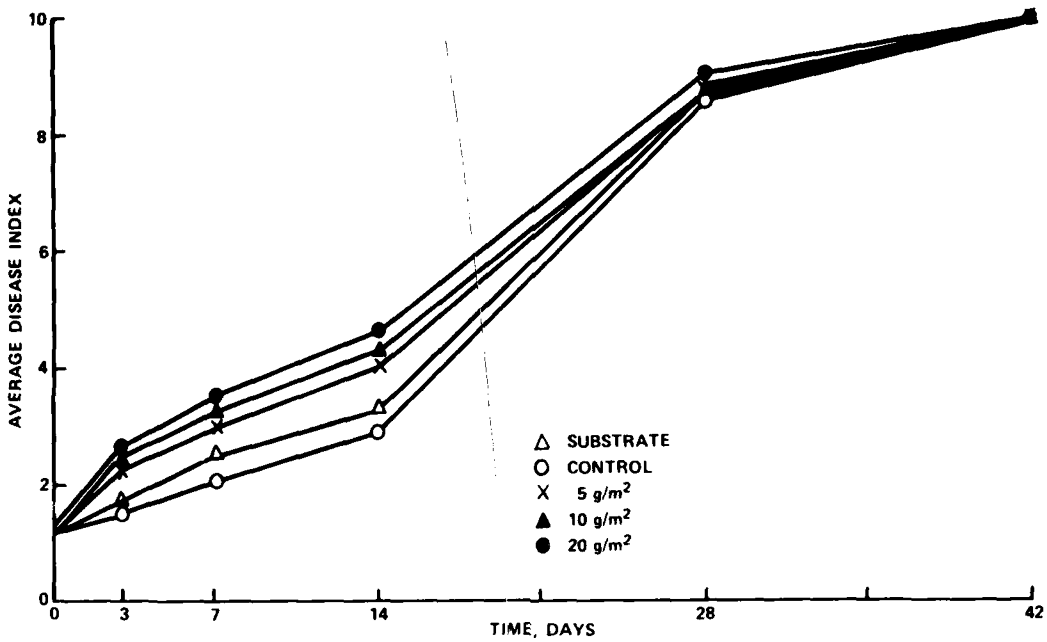

Figure 12. The ADI values for original leaves in tanks receiving *C. rodmanii* as compared to control tanks in the spring study

b. New leaves. The ADI values for new leaves were consistently higher in treated tanks than in the controls (Figure 13). However, the observed differences were not statistically significant (Table 5). There were no significant differences in the number of new leaves per plant. Lesions produced through secondary infection by *C. rodmanii* were common on new leaves (Figure 14), and *C. rodmanii* was identified from tissues of new leaves collected on 1 June 1979 in the treated tanks. These data verified that the formulation contained viable propagules that produced sporulating mycelium in the original tissues, which then served as a source for the secondary infection of waterhyacinth tissues.

c. Daughter plants. Fourteen days after application, there were 10 to 24 percent fewer daughter plants in the treated tanks as compared to controls (Figure 15). However, ADI

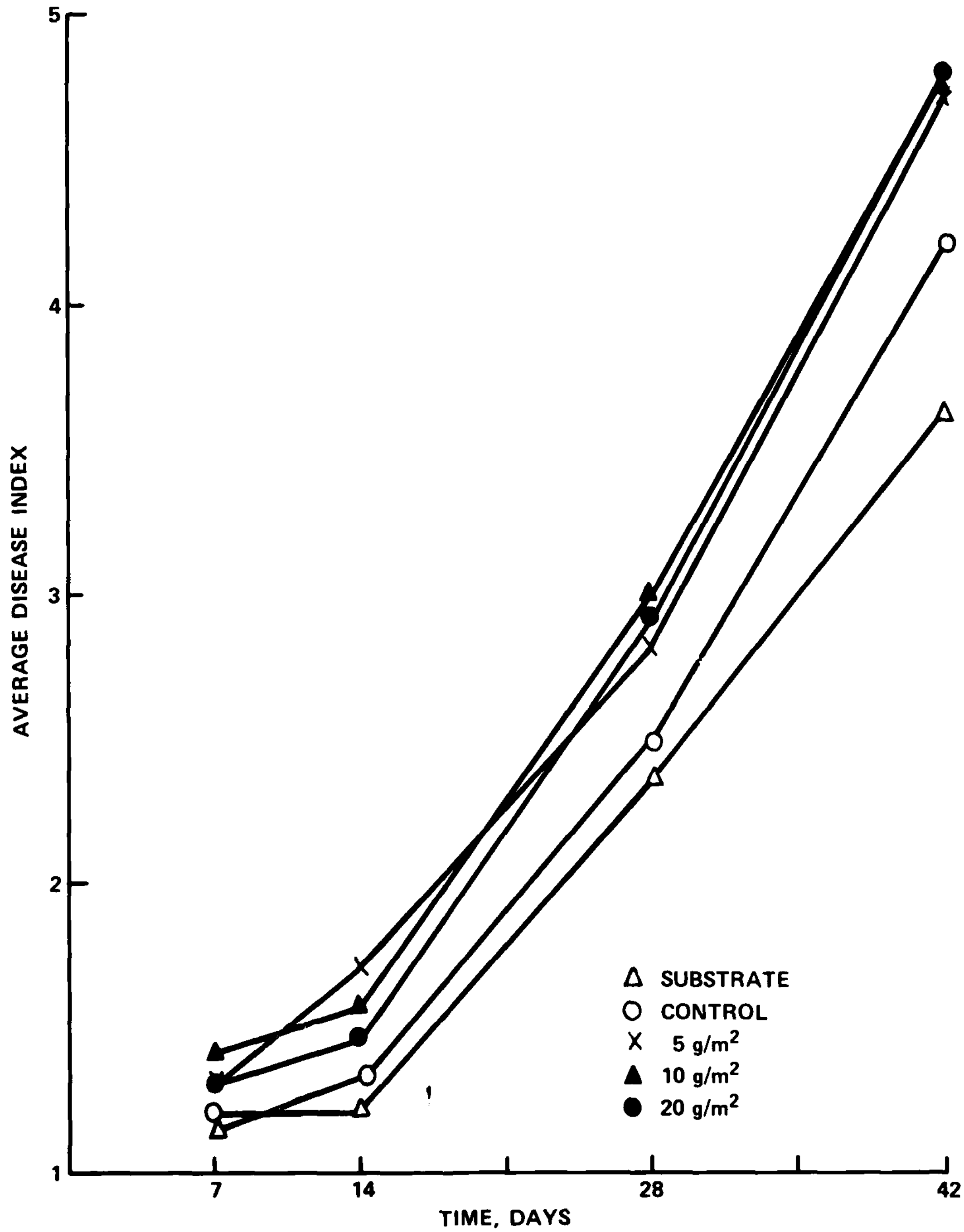

Figure 13. The ADI values for new leaves in tanks receiving C. rodmanii as compared to control tanks in the spring study

Figure 14. Secondary infection of new leaves of treated waterhyacinth plants by C. rodmanii

values for daughter plants in treated tanks at 14 days posttreatment were not significantly greater than for daughter plants in control tanks (Table 6). These tissues did not receive direct application of the formulation, and 2 weeks was insufficient time for the infected tissues to produce conidia and achieve infection of new tissue (Conway 1976b). Therefore, the difference in the number of daughter plants was presumably due to sufficient disease stress on the original plants to limit the production of daughter plants in treated tanks.

27. After 28 days posttreatment, ADI values for daughter plants were significantly higher in the treated tanks than in the control tanks (Figure 16 and Table 6). However, there were no significant differences in the number of daughter plants (Figure 15). By this time, the tanks had become densely packed with plants, and further growth was manifested in the vertical growth of the existing leaves and tissues rather than in daughter plant production.

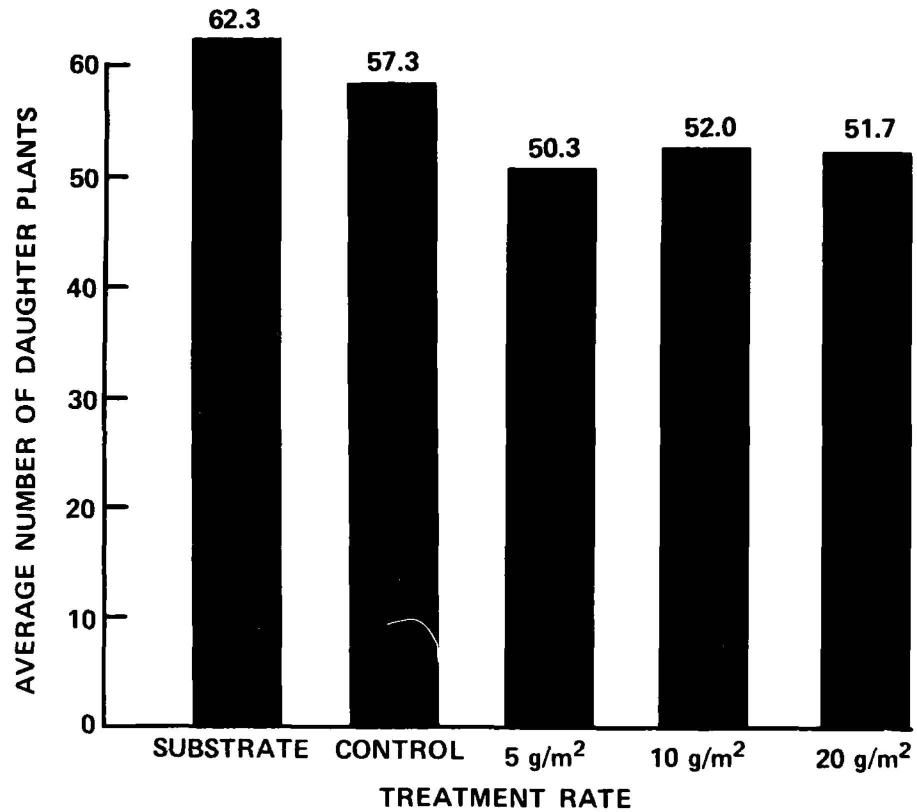

Figure 15. Comparison of average numbers of daughter plants among treatments at 14 days after application (spring study)

28. Forty-two days after application, there were no significant differences in ADI values of daughter plants between treated and control tanks (Table 6). This was due to an increase in ADI values in control tanks.

29. There were no significant differences in the number of dead leaves, plant height, root length, or biomass between treated and control tanks at any time during the study.

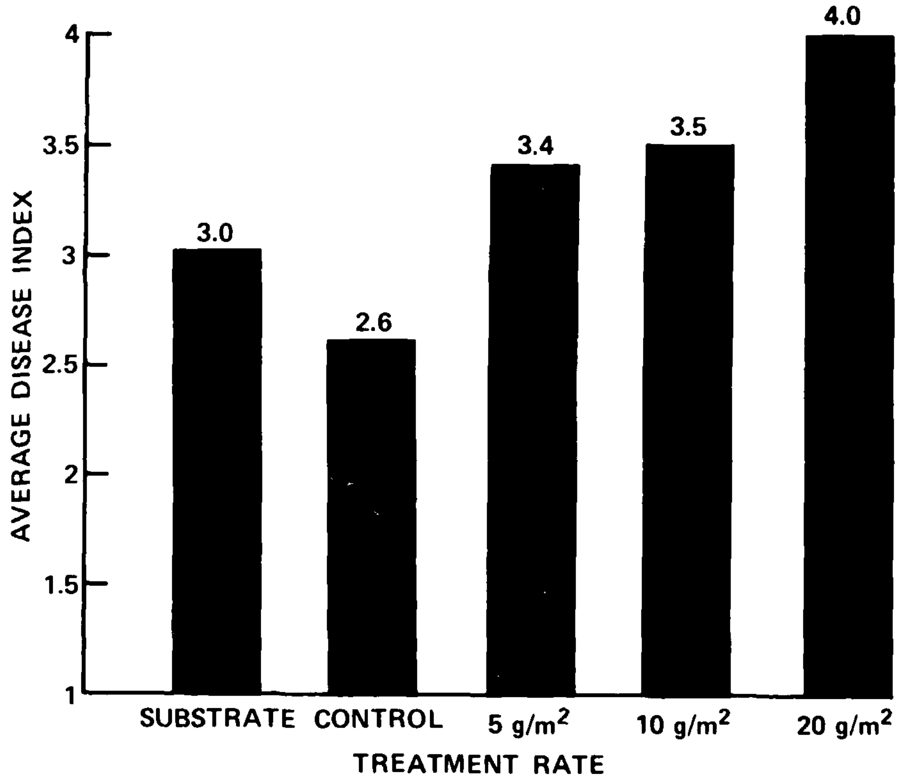

Figure 16. Comparison of ADI values of daughter plants among treatments at 28 days after application (spring study)

PART III: FALL STUDY

Procedure

Experimental unit

30. The same basic experimental design was followed in the fall study that was employed for the spring study (paragraph 8). The test tanks remained in place for the fall study. The plastic linings were replaced and the tanks were refilled. Nutrient solution was added to the water in the tanks at 312 mg/ℓ per tank. Water levels and pH were maintained as in the spring study.

Pretreatment data collection

31. Fifteen waterhyacinth plants from a greenhouse were placed in each tank on 2 October 1979 (Table 7). Six plants were tagged as in the spring study, but all 15 plants were left in the tanks to simulate denser field populations of waterhyacinth normally encountered during the fall.

Formulation and application

32. The Abbott Laboratories' formulation of C. rodmanii, containing 4×10^6 CFU/g, was applied at rates of 1, 2.5, 5, and 10 g/m^2 on 10 October (Table 7). Since no significant differences between the treated and untreated controls were observed in the spring study, only an untreated control was used.

33. The same procedure was used to apply the formulation as was used in the spring study (paragraph 16). The tanks were again covered with plywood for the first 24-hr period following application.

Posttreatment data collection

34. Biological parameters monitored were the same as for the spring study (paragraph 18), except that plant height was excluded because no significant differences in plant height among treatments were observed in the spring study. Color photographs were taken of tanks where significant effects were noted. Disease damage was assessed as in the spring test (paragraph 19).

Data analysis

35. Data were analyzed using the same statistical approach and analytical procedures that were used during the spring study (paragraph 20).

Results

Growth of control plants

36. An average of 3.5 new leaves was produced on the original plants during the 6-week test period (Table 8). This was approximately 39 percent of the number produced on original plants during the spring test. An average of three daughter plants (Table 8) was produced by each of the originally tagged plants (15 per tank), which was approximately 10 percent of the number produced during the spring study. Since there were no significant differences between the treated and control tanks, the decrease in production of new leaves and daughter plants was attributed to the environmental conditions present during the fall season and natural senescence that occurred during this season.

Initial infection

37. A 3-day posttreatment data collection was not conducted for the fall study. However, observations revealed that the reddish-brown blemishes on the surface of the treated plants were not as abundant as occurred during the spring study.

Pathogenicity

38. As in the spring study, the typical punctate leaf spots were present on the treated tissues 2 weeks after application. However, the disease symptoms increased in severity at a much slower rate than in the spring study. The ADI values were as follows:

a. Original tissue. The ADI values for original tissues in treated tanks were consistently higher than ADI values of original tissues in control tanks throughout the study (Figure 17). At 6 days posttreatment, the ADI values in tanks treated with 10, 5, and 2.5 g/m^2 of the *C. rodmanii* formulation were significantly higher than ADI values in

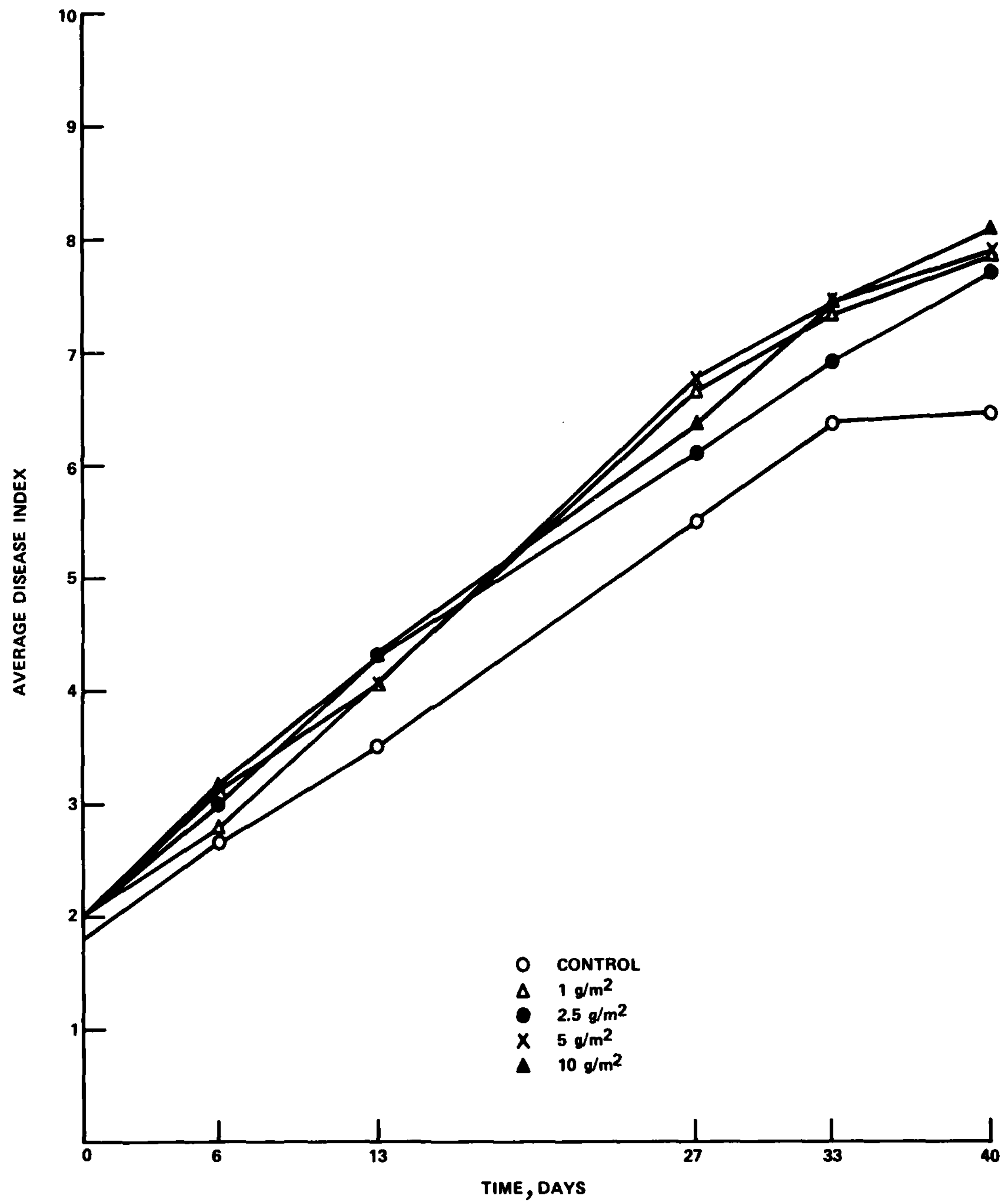

Figure 17. Comparison of ADI values for original tissues among treatments (fall study)

tanks treated at the 1-g/m^2 rate and the controls (Table 9). At 13 days posttreatment, the ADI values for tanks treated at rates of 10, 2.5, and 1 g/m^2 were significantly higher than for the other treatment rates. At 27 and 33 days post-treatment, the ADI values for the 10-, 5-, and 1-g/m^2 treatment rates were significantly higher than for the 2.5 g/m^2 and control tanks. On the last sampling date (40 days post-treatment), ADI values for all test tanks treated with the formulation were significantly higher than for the control tanks (Figure 18). A fall application rate of 1 g/m^2 (4 $\times 10^6$ CFU/m^2) of this formulation of _C. rodmanii_ was determined to be sufficient to achieve adequate infection of waterhyacinth.

b. New leaves. As for the original tissues, the ADI values for new leaves in the tanks treated with the _C. rodmanii_ formulation were consistently higher than the control tanks, except for 33 days posttreatment (Figure 19). At 6 days posttreatment, ADI values in tanks treated with 5 and 2.5 g/m^2 of the formulation were significantly higher than ADI values for the 1- and 10-g/m^2 rates and control. However, at 40 days posttreatment, only the ADI values for the 1-g/m^2 rate were significantly higher than the controls (Table 10). There were no significant differences in the number of new leaves between treatments during the test period.

c. Daughter plants. There were no significant differences in the ADI values of daughter plants until 40 days post-treatment (Table 11). At that time, ADI values for daughter plants in the tanks treated with 10, 5, and 2.5 g/m^2 were significantly higher than for the 1-g/m^2 rate. There were no significant differences in the number of daughter plants.

39. There were no significant differences among treatments in the number of dead leaves per plant.

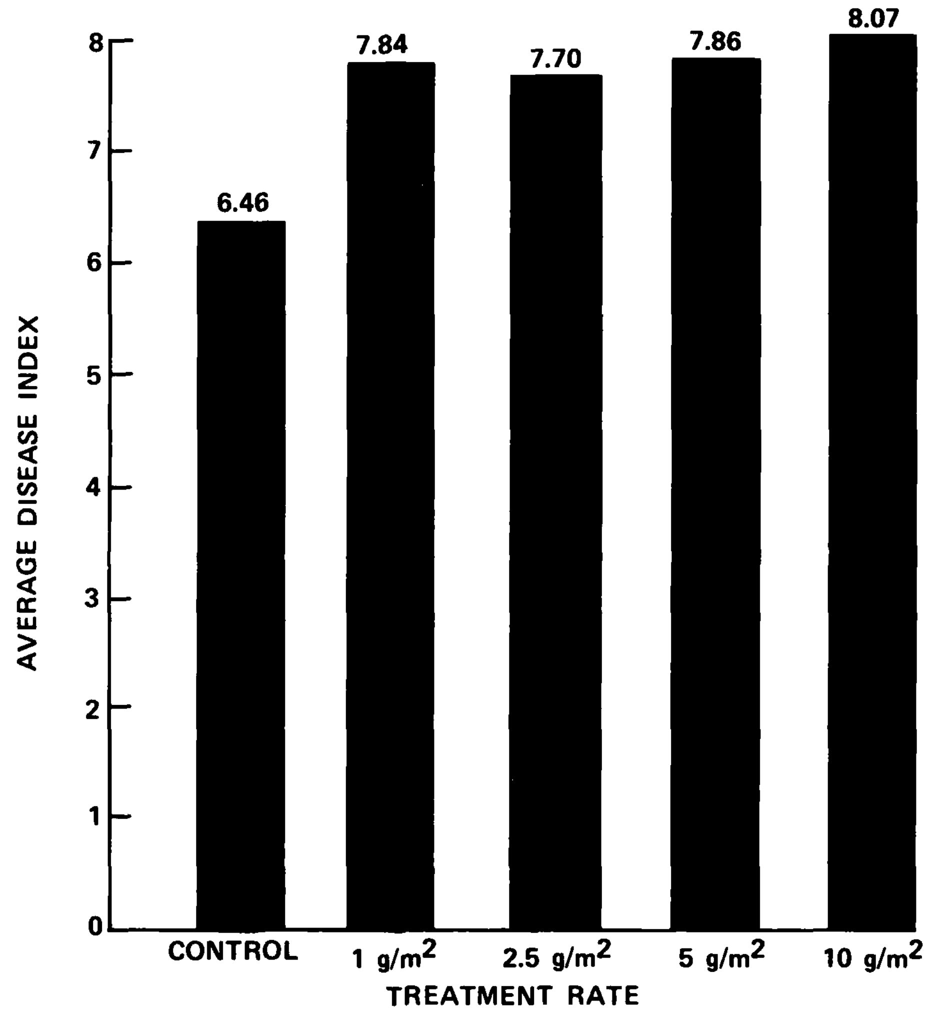

Figure 18. Comparison of ADI values of original tissues among treatments at 40 days posttreatment (fall study)

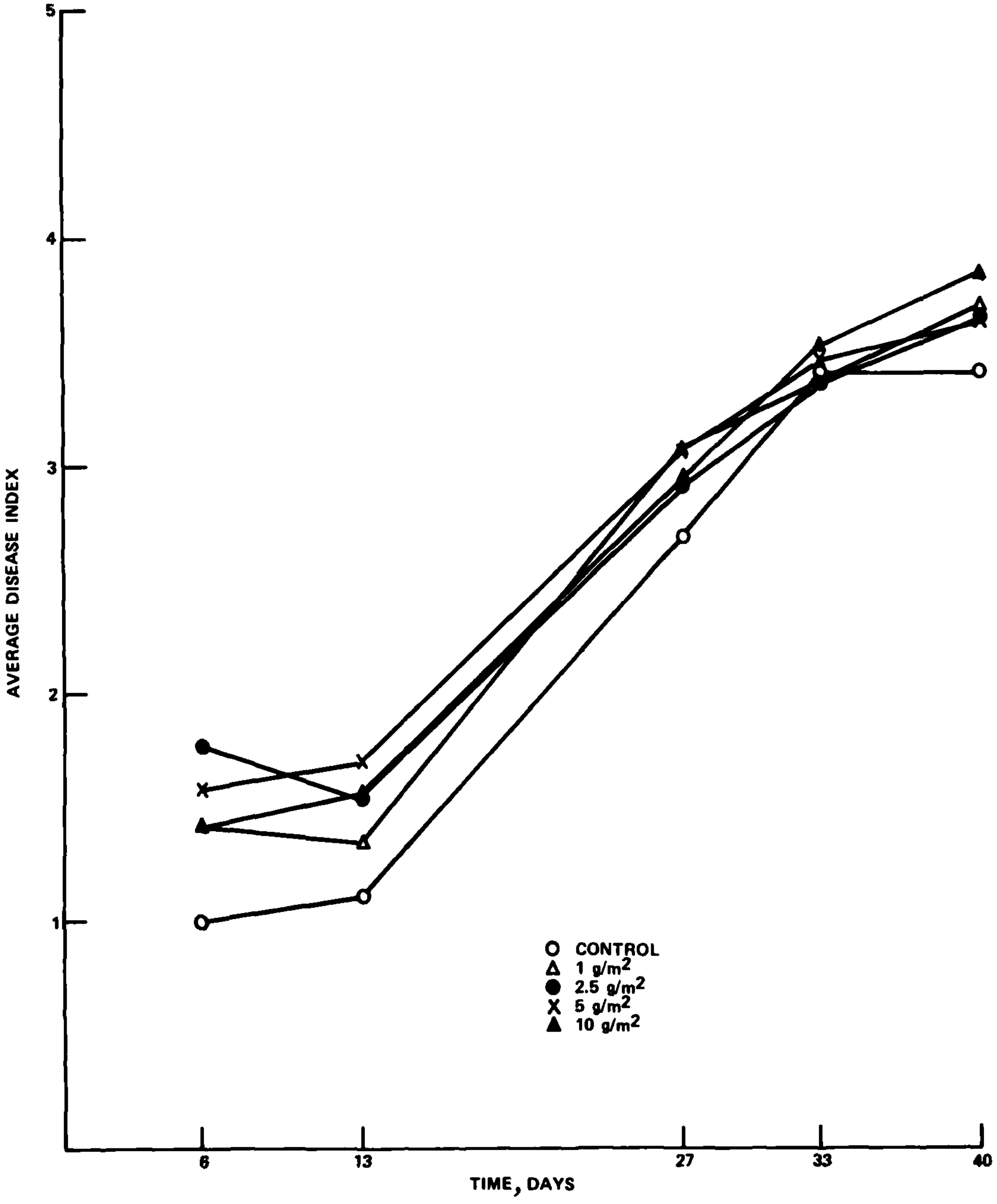

Figure 19. Comparison of ADI values for new leaves among treatments (fall study)

PART IV: DISCUSSION

40. In both the spring and fall studies, the lowest treatment rates (5×10^6 CFU/m^2 and 4×10^6 CFU/m^2, respectively) of the formulation produced significantly greater infection and disease development than the controls, but were not significantly different than the higher treatment rates. Therefore, treatment rates of 5×10^6 CFU/m^2 for a spring application and 4×10^6 CFU/m^2 for a fall application should produce a heavy infection of waterhyacinth.

41. These promising results were obtained even though the test design significantly favored waterhyacinth. All plants used for the study were healthy and free of disease and insect stress at the initiation of the studies. By periodically adding nutrients to the tanks and adjusting the pH of the water, optimal growth conditions were provided for the waterhyacinth. Except for a brief period during the fall study, temperatures were well within the range necessary for the growth of waterhyacinth. The only real advantage afforded *C. rodmanii* was the covering of the tanks during the initial 24 hr after application. However, the tanks were not covered to enhance infection by the pathogen, but to ensure that rainfall did not wash the inoculum off the plants.

42. Based on the results of these and other studies (Freeman et al. 1976), it was concluded that the *C. rodmanii* formulation could successfully infect waterhyacinth in either the spring or the fall. The slower growth rate of waterhyacinth in the fall definitely favored *C. rodmanii*, primarily because a greater percentage of the plant tissues were physiologically mature and were more susceptible to development of the disease process. The major disadvantage of the fall application was that less time was available for development of the pathogen population to the level required for achieving control of waterhyacinth. The amount of inoculum lost during the following winter season was not known. Whether or not sufficient loss of inoculum occurred during the winter season to negate the benefits afforded by a fall application was not determined.

43. On the other hand, application of *C. rodmanii* in the early

spring was advantageous because the pathogen was afforded the entire growing season to develop to the level required for control of waterhyacinth. However, Martyn (1977) reported that because of their higher phenoloxidase activity, the juvenile waterhyacinth plants were more resistant to infection by some pathogens, especially Acremonium zonatum (Sawada) Gams. If such a system also affected C. rodmanii, the establishment of C. rodmanii on waterhyacinth with a spring application would be more difficult. However, the 5×10^6-CFU/m^2 treatment rate for the spring study produced heavy infection of waterhyacinth, which suggested that C. rodmanii was not greatly affected by the natural disease resistance system in waterhyacinth.

44. Although the ability to achieve adequate infection of waterhyacinth by a spring application of C. rodmanii was demonstrated, it was not determined whether significant reduction in the waterhyacinth population would occur in the year of application. Due to the rapid growth rate of waterhyacinth, development of the C. rodmanii population lagged behind the normal growth rate of waterhyacinth in late spring and summer months. In addition, high temperatures that occurred from June to September produced a decline in activity of the pathogen. Therefore, the anticipated sequence of events that would be expected to occur from a spring application of C. rodmanii is as follows:

a. Cercospora rodmanii would become established on waterhyacinth and proliferate during the spring months.

b. If control was not achieved by June, there would be a significant decline in C. rodmanii activity, but it would persist on senescent waterhyacinth leaves in the understory throughout the summer months.

c. When the growth of waterhyacinth decreased in the fall and cooler temperatures favored C. rodmanii activity, the effects of the pathogen on waterhyacinth would increase until the colder, winter months.

d. Cercospora rodmanii would maintain itself in the dead and dying tissues of the floating mat during the winter. As many as 3 years would be required to achieve the maximum

impact of *C. rodmanii* on waterhyacinth (Addor 1977). The time required to produce the desired level of control would be influenced by climatic conditions, vigor of the waterhyacinth, and presence of other organisms (e.g. the weevil *Neochetina eichhorniae* (Warner), and the moth *Sameodes albiguttalis* (Warren)) that also stress waterhyacinth.

45. Considering that the 1-g/m^2 treatment rate (4×10^6 CFU/m^2) resulted in adequate infection of waterhyacinth in a fall application, the *C. rodmanii* formulation could be applied at levels in the same order of magnitude as used for herbicide application. The 1-g/m^2 treatment rate was equivalent to 10,000 g/ha (approximately 9 lb/acre). At this rate, conventional herbicide application equipment could be used to apply the formulation (Theriot, Sanders, and Theriot 1981). Although the economics of the formulation have not been established, the application of such low rates would make the *C. rodmanii* formulation competitive with chemical herbicides, considering the long-term control afforded by the use of the pathogen.

46. The potential of this formulation of *C. rodmanii* for infecting waterhyacinth was clearly demonstrated. The formulation must be evaluated more extensively under true field conditions in areas of operational interest to determine treatment rates, methods of application, and time of application to achieve its maximum impact on the waterhyacinth population. Further studies should be conducted to determine if the level of infectivity and pathogenicity of the *C. rodmanii* formulation could be enhanced through the use of surfactants and/or an added nutrient source.

PART V: CONCLUSIONS

47. Based on the results of these studies, the following conclusions can be drawn:

- a. The Abbott Laboratories' formulation of *C. rodmanii* was infectious on waterhyacinth, even under less than optimal environmental conditions and on plants of high vigor.
- b. Hyphal tissues produced by the original inoculum proliferated in waterhyacinth tissues.
- c. Secondary infection of new waterhyacinth tissues (new leaves and daughter plants) occurred from conidia produced on the originally infected plants.
- d. Vegetative reproduction of a waterhyacinth population was reduced following a spring application of the formulation.
- e. A treatment rate of 5×10^6 CFU/m^2 of the formulation achieved a heavy infection of *C. rodmanii* on waterhyacinth in a spring application.
- f. A treatment rate of 4×10^6 CFU/m^2 produced a heavy infection on waterhyacinth in a fall application.

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Table 1

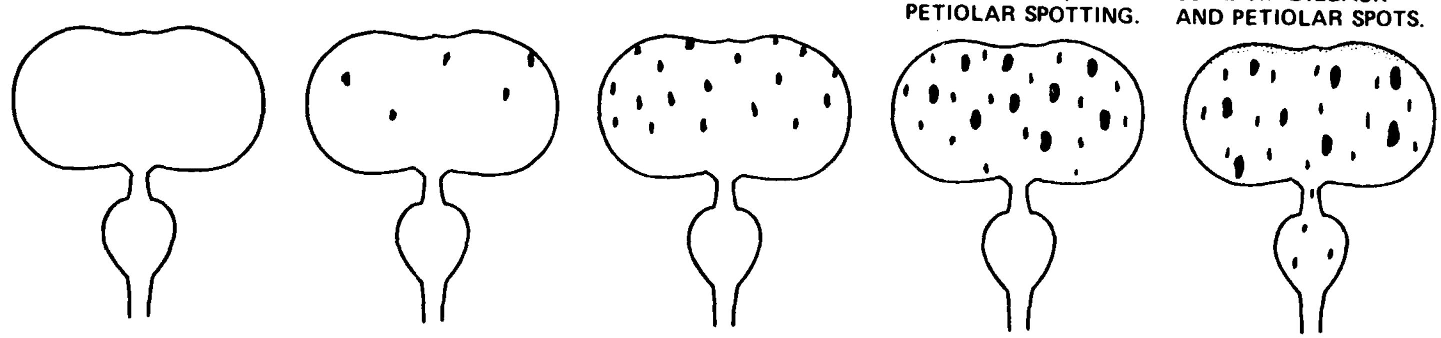
Schedule of Events for the Spring Study

Activity	Date
Establishment of test tanks	15 March 1979
Nutrient solution added to tanks	16 March 1979
Waterhyacinths placed in tanks	19 March 1979
Pretreatment data collection	12 April 1979
Application of *C. rodmanii*	13 April 1979
Nutrient solution added to the tanks	27 April 1979
Posttreatment data collection	
3 days	16 April 1979
7 days	20 April 1979
14 days	27 April 1979
28 days	11 May 1979
42 days	25 May 1979

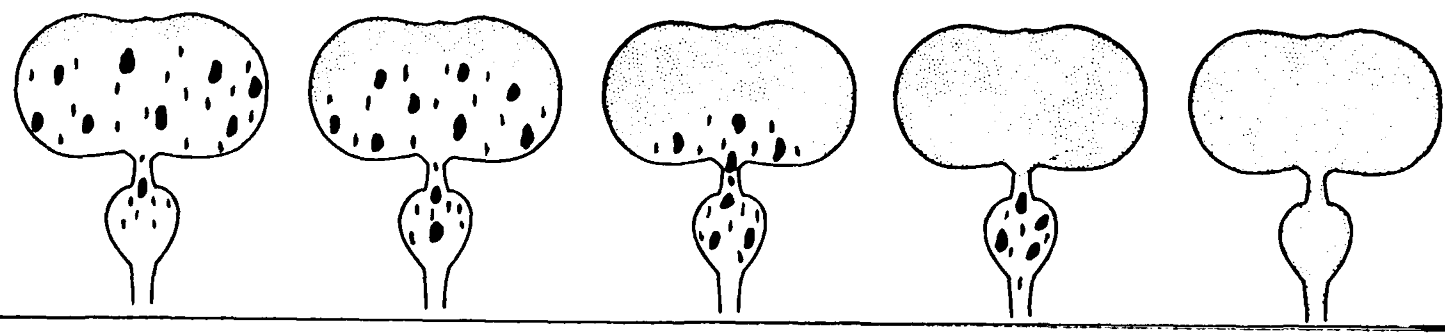
Table 2

Disease Index for Damage to Leaves of Waterhyacinth by C. rodmanii*

NUMERICAL RATING	1	2	3	4	5
SYMPTOMS	NO SPOTS ON LEAF OR PETIOLE.	1-4 SPOTS ON LEAF, NO PETIOLAR SPOTTING.	LESS THAN 25% OF LEAF SURFACE WITH SPOTS, NO COALESCENCE OR PETIOLAR SPOTTING.	LESS THAN 50% OF LEAF SURFACE WITH SPOTS, SOME COALESCENCE, NO PETIOLAR SPOTTING.	LESS THAN 25% OF LEAF SURFACE WITH SPOTS, COALESCENCE, SOME TIP-DIEBACK AND PETIOLAR SPOTS.



NUMERICAL RATING	6	7	8	9	10
SYMPTOMS	LESS THAN 50% OF LEAF SURFACE WITH SPOTS, COA-LESCENCE, 10% TIP-DIEBACK, PETIOLE SPOTTING.	LESS THAN 75% SPOTS, COALESCENCE, (30%) TIP-DIEBACK, IN-CREASING PETIOLAR SPOTTING.	GREATER THAN 75% SPOTS, COALESCENCE, (60%) TIP-DIEBACK, COALESCING SPOTS ON PETIOLE.	DEAD LEAF BLADE, PETIOLE GREEN, BUT HEAVILY SPOTTED.	DEAD LEAF BLADE AND PETIOLE (SUBMERGED).



* Conway and Freeman (1976).

Table 3

Average Vegetative Reproduction and Growth Rates of Waterhyacinth During the Spring Study

Date	Posttreatment Period, days	Original Leaves per Plant	New Leaves per Plant	Daughter Plants per Plant	Height cm	Biomass per Tank kg	Root Length per Plant cm
12 April 1979	0	5.3	0.0	0.0	13.5	0.97	17.2
16 April 1979	3	5.3	1.0	0.0	12.5	--	--
20 April 1979	7	5.3	2.0	5.5	12.0	--	--
27 April 1979	14	5.3	3.8	9.1	18.9	--	--
11 May 1979	28	1.8	6.8	19.0	25.1	--	--
25 May 1979	42	0	9.0	29.0	30.3	--	--
31 May 1979	48	--	--	--	--	14.15	18.3

Table 4

Average Disease Index per Leaf for Original Waterhyacinth Tissue After Treatment with C. rodmanii During the Spring Study

Treatment Rate, g/m^2	Time, days					
	0	3	7	14	28	42
0 (control)	1.19 a*	1.57 a	2.07 a	2.88 a	8.71 a	10.00 a
0 (substrate)	1.21 a	1.77 a	2.52 a	3.37 a	8.76 a	9.92 a
5	1.17 a	2.21 b	2.98 b	4.05 b	8.99 a	10.00 a
10	1.18 a	2.56 b	3.35 b	4.28 b	8.92 a	10.00 a
20	2.27 a	2.68 b	3.53 b	4.67 b	9.29 a	10.00 a

* Means within a column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test.

Table 5

Average Disease Index per Leaf for New Leaves of Original Waterhyacinth Plants After Treatment with C. rodmanii During the Spring Study

Treatment Rate, g/m^2	Time, days					
	0	3	7	14	28	42
0 (control)	NA*	NA	1.17 a**	1.33 a	2.49 a	4.21 a
0 (substrate)	NA	NA	1.21 a	1.27 a	2.36 a	3.62 a
5	NA	NA	1.31 a	1.72 a	2.80 a	4.71 a
10	NA	NA	1.44 a	1.57 a	2.97 a	4.74 a
20	NA	NA	1.32 a	1.48 a	2.91 a	4.78 a

* NA = Not applicable because there were no new leaves on this date.
** Means within a column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test.

Table 6

Average Disease Index per Leaf for Daughter Plants After Treatment with C. rodmanii During the Spring Study

Treatment Rate, g/m^2	Time, days					
	0	3	7	14	28	42
0 (control)	NA*	NA	1.00 a**	1.50 a	2.60 a	4.60 a
0 (substrate)	NA	NA	1.10 a	1.47 a	2.96 a	4.40 a
5	NA	NA	1.07 a	1.93 a	3.43 b	4.47 a
10	NA	NA	1.00 a	1.53 a	3.47 b	4.83 a
20	NA	NA	1.07 a	1.80 a	3.97 b	4.07 a

* NA = Not applicable because there were no daughter plants.

** Means within a column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test.

Table 7

Schedule of Events for the Fall Study

Activity	Date
Establishment of test tanks	2 October 1979
Nutrient solution added to tanks	2 October 1979
Pretreatment data collection	9 October 1979
Application of formulation	10 October 1979
Posttreatment data collection	
6 days	16 October 1979
13 days	23 October 1979
27 days	7 November 1979
33 days	13 November 1979
40 days	20 November 1979

Table 8

Average Vegetative Reproduction and Growth Rates of Waterhyacinth During the Fall Study

Date	Posttreatment Period, days	Original Leaves per Plant	New Leaves per Plant	Daughter Plants per Plant
9 October 1979	0	4.1	0	0
16 October 1979	6	4.1	1.0	0
23 October 1979	13	4.1	2.3	2.5
7 November 1979	27	3.9	3.2	2.8
13 November 1979	33	3.5	3.5	3.0
20 November 1979	40	3.1	3.5	3.0

Table 9

Average Disease Index per Leaf for Original Waterhyacinth Plants After Treatment with C. rodmanii During the Fall Study

Treatment Rate, g/m^2	Time, days					
	0	6	13	27	33	40
0 (control)	1.79 a*	2.65 a	3.51 a	5.49 a	6.37 a	6.46 a
1	1.99 a	2.76 ac	2.17 b	6.64 b	7.33 b	7.84 b
2.5	2.02 a	2.99 bc	4.29 b	6.10 ab	6.91 ab	7.70 b
5	2.09 a	3.01 bc	4.07 ab	6.75 b	7.43 b	7.86 b
10	2.06 a	3.13 b	4.32 b	6.38 b	7.43 b	8.07 b

* Means within a column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test.

Table 10

Average Disease Index per Leaf for New Leaves of Original Waterhyacinth Plants After Treatment with C. rodmanii During the Fall Study

Treatment Rate, g/m^2	Time, days					
	0	6	13	27	33	40
0 (control)	0	1.00 a*	1.11 a	2.69 a	3.40 a	3.41 a
1	0	1.42 ab	1.34 ab	3.08 b	3.39 a	3.70 b
2.5	0	1.76 b	1.54 b	2.91 ab	3.37 a	3.65 ab
5	0	1.58 b	1.70 b	3.01 b	3.46 a	3.63 ab
10	0	1.42 ab	1.56 b	2.95 b	3.52 a	3.84 ab

* Means within a column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test.

Table 11

Average Disease Index per Leaf for Daughter Plants After Treatment with C. rodmanii During the Fall Study

Treatment Rate, g/m^2	Time, days				
	0	13	27	33	40
0 (control)	0 a	1.24 a*	2.23 a	2.79 a	2.82 a
1	0 a	1.11 a	2.39 a	2.68 a	2.98 ac
2.5	0 a	1.37 a	2.41 a	2.87 a	3.19 bc
5	0 a	1.19 a	2.30 a	2.75 a	3.13 bc
10	0 a	1.28 a	2.37 a	2.83 a	3.27 b

* Means within a column followed by the same letter are not significantly different ($p < 0.05$) using Duncan's Multiple Range Test.

In accordance with letter from DAEN-RDC, DAEN-ASI dated 22 July 1977, Subject: Facsimile Catalog Cards for Laboratory Technical Publications, a facsimile catalog card in Library of Congress MARC format is reproduced below.

Theriot, Edwin A.
  Evaluation of a formulation of Cercospora rodmanii for infectivity and pathogenicity of waterhyacinth : final report / by Edwin A. Theriot, Russell F. Theriot, Dana R. Sanders, Sr. (Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station). -- Vicksburg, Miss. : The Station ; Springfield, Va. : available from NTIS, [1981].
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